A METHOD OF EXTRACTING AND ISOLATING MINOR COMPONENTS FROM VEGETABLE OIL

FIELD OF THE INVENTION

The present invention relates to a method of extracting and isolating minor components such as carotenes, tocols in the form of tocopherols and tocotrienols and hydrocarbons such as squalene and phytosterols from vegetable oils, preferably but not necessarily from palm oil.

BACKGROUND OF THE INVENTION

Crude palm oil (CPO) contains about 1% minor components which include carotenes, tocols in the form of tocopherols and tocotrienols and hydrocarbons such as squalene and phytosterols. These minor components are also present in oil that is obtained from the palm pressed fiber in a much higher concentration.

Carotenes, tocols and squalene have been known for long to exhibit antioxidant properties and they are widely used in pharmaceuticals as supplements, nutraceuticals as well as fine chemicals. Squalene, which is widely found in shark liver oil is also present in palm oil and it is a valuable constituent in cosmetics as well as food supplements. Sterols can be used as steroid derivatives in pharmaceuticals. The major sterol present in palm oil, β-sitosterol has been shown to possess hypocholesterolemic effect.

Several methods have been developed to extract these valuable minor components from vegetable oils namely, extraction by saponification which have been disclosed in US Patents No. 2,460,796, 2,572,467 and 2,652,433 as well as in UK Patent No. GB 567,682, iodine method, urea process and extraction by Fuller's earth or activated carbon which have been disclosed in UK Patents No. 691,924 and 1,563,794 as well as in US Patent 2,484,040.

Further extraction methods which are known to artisans in the field of the invention are solvent extraction which has been disclosed in US Patent 2,432,021, molecular distillation, solvent partitioning and adsorption which has disclosed in US Patent 5,157132 and UK Patent No. 2,160,874.

SUMMARY OF THE INVENTION

The present invention relates a method of extracting and isolating minor components such as carotenes, tocols in the form of tocopherols and tocotrienols and hydrocarbons such as squalene and phytosterols from vegetable oils, preferably but not necessarily from palm oil. The said method process comprises the steps of a) esterification of the oil with an alcohol, said esterification provides a mixture of a glycerol, fatty acids esters and the minor components, b) collection of the esters phase containing the minor components from glycerol, c) distillation of the esters phase, said distillation provides a concentrate of squalene, carotenes, tocols and sterols at a temperature from room temperature to 200°C and under a pressure from 0 to 150 mm Torr, d) dilution of the concentrate in a non-polar solvent or a mixture of non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar, e) adsorption of the concentrate obtained from step (c) on an adsorbent, f) extraction of the minor components from the concentrate obtained from step (e) by desorption of the minor components using a predetermined mixture of solvents which is a non-polar solvent or a mixture of non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar and g) desorption of the minor components by a predetermined mixture of solvents.

There is also provided a method for isolating individual carotene such as β-carotene, α-carotene, lycopene, phytoene and phytofluene, individual tocol such as α-, γ- and δ-tocopherol and tocotrienol and individual sterol such as β-sitosterol from vegetable oil under isocratic and isobaric conditions under a pressure between 0.2 – 1000 bar, the

method comprising the steps of a) adsorbing the oil to an adsorbent and b) desorbing the mixture obtained in step (a) a mixture of polar and non-polar solvents.

Also provided a method for isolating individual carotene such as β-carotene, α-carotene, lycopene, phytoene and phytofluene, individual tocol such as α-, γ- and δ-tocopherol and tocotrienol and individual sterol such as β-sitosterol from minor components such as squalene, carotenes, tocols and sterols obtained from a method which is conducted under an isocratic condition, the method comprising the steps of a) esterification/transesterification of the oil with an alcohol, said esterification provides a mixture of a glycerol, fatty acids esters and the minor components, b) collection of the esters phase containing the minor components from glycerol, c) distillation of the esters phase, said distillation provides a concentrate of squalene, carotenes, tocols and sterols at a temperature from room temperature to 200°C and under a pressure from 0 to 150 mm Torr, d) dissolution of the concentrate in a non-polar solvent or a mixture of non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar, e) adsorption of the concentrate obtained from step (c) on an adsorbent, f) extraction of the minor components from the concentrate obtained from step (e) using a predetermined mixture of solvents which is a non-polar solvent or a mixture of non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar and g) desorption of the minor components by a predetermined mixture of solvents characterised in that the minor components obtained from the above method undergo a further extraction under isocratic and isobaric conditions under a pressure between 0.2 – 1000 bar, the extraction comprising

the steps of 1) adsorbing the minor components to an adsorbent and 2) desorbing the mixture obtained in step (a) a mixture of polar and non-polar solvents.

The present invention consists of certain novel features and a combination of parts hereinafter fully described and particularly pointed out in the appended claims, it being understood that various changes in the details may be without departing from the scope of the invention, or sacrificing any of the advantage of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to a method of extracting and isolating minor components such as carotenes, tocols in the form of tocopherols and tocotrienols and hydrocarbons such as squalene and phytosterols from vegetable oils, preferably but not necessarily from palm oil. Hereinafter, this specification will describe the present invention according to the preferred embodiments. However, it is to be understood that limiting the description to the preferred embodiments of the invention is merely to facilitate discussion of the present invention and it is envisioned that those skilled in the art may devise various modifications and equivalents without departing from the scope of the appended claims.

The vegetable oil is esterified with lower alcohols such as methanol, ethanol, isopropanol and buthanol to give a mixture of glycerol, fatty acids alkyl esters, carotenes, tocols, squalene and sterols. The minor components are contained in the oil phase of the mixture which is then separated from glycerol.

The esters phase containing minor components is subjected to distillation under or without vacuum in which it is heated to not more than 200^0C and distilled at a pressure less than 150 mm Torr to retain as much of the minor components in the concentrate. Upon distillation, the fatty acid alkyl esters are removed from the mixture, leaving a concentrate rich in carotenes, tocols, squalene and sterols.

The concentrate collected is dissolved in a non-polar solvent or a mixture of non-polar solvent and a polar solvent with the non-polar solvent being the major constituent and adsorbed on a selected adsorbent. Different types of adsorbents such as normal-phase silica gel, reversed-phase (particularly C18) silica gel or neutral alumina may be employed. The minor components are extracted and desorbed from the adsorbents by a certain mixture of solvents depending on the type of adsorbent in use. Solvents that are used to desorb as well as to carry the minor components may consist of polar solvents such as ethanol, isopropanol, methanol or buthanol, non-polar solvents such as hexane, heptane, ethyl acetate, isooctane or cyclohexane.

The extraction or fractionation is carried out in an isocratic manner which is an improvement over US Patent No. 6,072,092 and UK Patent No. GB 2218989 whereby solvents with different polarity are added one after another to desorb all the compounds which is rather time and labour consuming, most important of all it is not technically and economically viable for commercial production. Also, US Patent No. 6,072,092 has only disclosed recovery of carotenes from palm oil in all the examples whereas the present invention disclose recovery of carotene, vitamin E, sterols and squalene in a single adsorption/desorption process.

The desorption of the minor components from the adsorbents is carried out through a column whereby it is subjected to an applied pressure between 0.2 to 50 bar resulting in faster and more efficient separation.

The above-described process can also be adopted for the isolation of individual carotene (i.e. β-carotene, α-carotene, lycopene, phytoene, phytofluene), tocol (α-, γ- and δ-tocopherol and tocotrienol) and sterol (β-sitosterol) from vegetable oils. Isolation of such individual compounds is an extension of the first extraction or from any sources that are rich in carotenes, tocols or sterols wherein operating pressure of the said process may be in the range of 0.2 – 1000 bar. Isobaric and isocratic conditions are applied.

Each resulting fractions are then subjected to a second extraction wherein individual carotene (α-carotene, β-carotene, lycopene, phytoene, phytofluene), tocol (α-, γ- and δ-tocopherol and tocotrienol) and β-sitosterol can be isolated and recovered. The second method consists of adsorbing the feed to an adsorbent such as normal-phase silica gel, reversed-phase silica gel or neutral alumina, preferably reversed-phase C18 silica gel and desorbed using a mixture of polar and non-polar solvent. The source of the individual carotenes, tocols and sterols is obtained from vegetable oil or concentrate or phytonutrients rich-fractions. The preferred vegetable oil will be palm oil.

The said process is an isocratic and isobaric process wherein the operating pressure may range from 0.2 – 1000 bar. Solvents used for desorption may consist of non-polar solvents such as hexane, heptane, ethyl acetate, isooctane or cyclohexane and polar solvents such as ethanol, isopropanol, methanol or buthanol.

It should be appreciated that the isolation of individual carotene, tocol and sterol by the said process can be carried out using the resulting fractions extracted from the concentrate or products from any sources that contains the said compounds.

Following is a description by way of examples of the recovery of carotenes, tocols, hydrocarbons such as squalene and sterols by different adsorbents.

Example 1

Crude palm oil methyl esters and palm oil minor components, derived from alcoholic esterification of crude palm oil with 1 to 2 % base catalyst and methanol is subjected to molecular distillation at a temperature less than 200^0C and pressure not more than 150 mm Torr to give concentrate with composition of 5% carotene, 0.5% tocols, 0.7% squalene and 0.7% sterols.

1.4 gram sample of the above concentrate is dissolved in 10 milliliters of hexane and added to the top of a normal-phase silica gel packed column of 12cm long and 4cm internal diameter. The feed to adsorbent ratio is 23g/kg. Mixture of hexane and isopropanol with hexane:IPA (99.5:0.5) is added to the column in which it is applied with not more than 1 bar pressure. Fractions are collected whereby squalene is the first to be eluted. This is followed by carotenes, tocols and last of all the phytosterols. Solvents in the collected fractions are rotavaporised. The concentration of squalene in the squalene fraction is determined by gas chromatography (GC) to be 7%, carotenes in the carotene fraction is determined by UV spectrometry at about 24%. The tocols are determined by HPLC on silica 60A column having the dimension 250mm X 46mm I.D. with fluorescent detector. The mobile phase is hexane:THF:IPA (1000:60:4). The concentration of tocols in tocols fraction is about 62%. GC determines the composition of phytosterols and there are about 29% of sterols in sterols fraction.

	Squalene	Carotene	Tocols	Sterols
Concentration in feed, %	0.7	5	0.5	0.7
Concentration in fraction, %	7	24	62	29
Recovery %	92	41	3	5

Example 2

1.4 gram concentrate obtained from the same manner with the same composition as Example 1 is dissolved in 10 millilitres hexane and added to the top of a 12cm long, 4 cm internal diameter of normal-phased silica gel packed column. Mixture of Hexane:IPA (99.5:0.5) is then added to the column with an applied pressure of less than 1 bar. Fractions are collected and the concentration of the minor components in their respective fractions is determined to be: carotenes 28% with 87% recovery, tocols 71% with 38% recovery, and sterols 6%.

	Carotene	Tocols	Sterols
Concentration in feed, %	5	0.5	0.7
Concentration in fraction, %	28	71	6
Recovery, %	87	38	5

Example 3

2.5 gram of concentrate obtained from the same manner as in Example 1 is dissolved in hexane and added to the top of a 12cm long 4cm internal diameter normal-phase silica packed column. Mixture of hexane:ethanol (99.5:0.5) is added with pressure less than 1 bar to elute the minor components. Fractions collected are determined for carotenes and

tocols content. 43% carotenes are recovered from a fraction with 45% carotenes. In the tocols fractions, the concentration of the tocols is determined to be 100% with 8% recovery from that particular fraction.

	Carotene	Tocols
Concentration in feed, %	5	0.5
Concentration in fraction, %	45	100
Recovery, %	43	8

Example 4

0.5 gram concentrate obtained from the same manner as in Example 1 is added to the top of a 7.5cm long, 4cm internal diameter reversed-phase C18 silica gel packed column. Ethanol:hexane (60:40) are added to the top of the column with pressure less than 1 bar to elute the minor components. However, the range of ethanol:hexane can be changed depending on any other conditions up to 90:10. Sterols and tocols are first to be eluted and their concentrations are 4% and 3% each. The recovery of sterols is 72% and tocols is 75%. Carotenes and squalene are eluted in later fractions and the concentration of carotenes in their enriched fraction is 27% and the concentration of squalene is 4% with 40% recovery.

	Squalene	Carotene	Tocols	Sterols
Concentration in feed, %	0.7	5	0.5	0.7
Concentration in fraction, %	4	27	3	4
Recovery, %	40	40	75	72

Example 5

1.3g concentrate obtained from the same manner as Example 1 is dissolved in hexane and added to the top of a 12cm long. 4cm internal diameter silica column. Mixture of hexane:ethanol (99.5:0.5) is added to the top of the column with pressure less than 1 bar to desorb the minor components. Fractions collected are determined with the following results:

	Squalene	**Carotene**	**Tocols**	**Sterols**
Concentration in feed, %	0.7	5	0.5	0.7
Concentration in fraction, %	24	1	3	29
Recovery, %	34	3	2	58

Example 6

1.5g concentrate obtained from the same manner as Example 1 is dissolved in hexane and added to the top of a 12cm long, 4cm internal diameter silica packed column. Mixture of hexane:IPA (99.5:0.5) is added to the top of the column with pressure less than 0.5 bar to desorb the minor components. Fractions collected are determined with the following results:

	Squalene	**Carotene**
Concentration in feed, %	0.1	7.9
Concentration in fraction, %	23	64
Recovery, %	71	60

The whole process uses less than 1.9 liters solvent and is completed in less than 30 minutes.

Example 7

1.6g concentrate obtained from the same manner as Example 1 is dissolved in hexane and added to the top of a 12cm long, 4cm internal diameter C18 column. Mixture of Ethanol:Hexane (60:40) is added to the top to desorb the minor components with pressure less than 1 bar. Composition of carotene recovered is 14% with 12% recovery.

Example 8

2.9g concentrate rich in vitamin E is added to the top of a 12cm long, 40mm internal diameter silica column. Hexane:ethyl acetate (80:20) is added to elute the individual vitamin E with pressure less than 0.5 bar. α-tocopherol (α-T) is the first to be eluted followed by α-Tocotrienol (α-T_3), γ-Tocopherol (γ-T), γ-Tocotrienol (γ-T_3) and last of all, the δ-Tocotrienol (δ-T_3). The purity of respective collected tocols are as follows:

	α-T	α-T_3	γ-T	γ-T_3	δ-T_3
Composition in feed, %	9	9	1	16	3
Composition in fraction, %	42	54	9	68	40
Recovery, %	24	28	41	21	64

Example 9

2.5g concentrate rich in vitamin E is loaded to a 12cm long, 40mm internal diameter silica column. Hexane:Ethyl Acetate (86:14) is added to elute the vitamin E with pressure less than 0.5 bar. The purity of the respective tocols collected are as follows:

	α-T	α-T_3	γ-T	γ-T_3	δ-T_3
Composition in feed, %	9	9	1	16	3
Composition in fraction, %	46	28	8	80	56
Recovery, %	33	22	32	30	49

Example 10

0.8g concentrate obtained from the same manner as Example 1 is dissolved in hexane and loaded to a 25cm long, 20cm internal diameter silica column. Mixture of hexane:IPA (98:2) is added to the column with pressure less than 1500psi to desorb the minor components. Squalene is the first to be collected followed by carotenes, vitamin E, sterols and last of all the unreacted diglycerides. The composition of the fractions are collected are as follows:

	Squalene	**Carotene**	**Vitamin E**	**Sterols**	**Diglycerides**
Composition in feed, %	1.5	2.6	1.9	0.9	2.6
Composition in fraction, %	4	50	18	3.4	20
Recovery, %	34	47	9	1	13

Example 11

1.6g concentrate obtained from the same manner as Example 1 is loaded into a 7cm long, 13cm internal diameter silica packed column. 100% hexane is added to the top to desorb the minor components with pressure less than 1 bar. First fraction collected consists of squalene while the second fraction consists of carotene. After the carotene fraction is collected, the polarity of the mobile phase is gradually increase using isopropanol started with 2 – 5% IPA in hexane. The later fraction collected consists of Vitamin E followed by sterols. The compositions and recovery of these fractions are as follows:

	Squalene	Carotene	Vitamin E	Sterols
Composition in feed, %	1.5	6.8	2.6	0.4
Composition in fraction, %	31	50	1.4	1.1
Recovery, %	80	39	26	25

Example 12

A 40mm I.D. column is packed with 6.6cm length normal phase silica and top up with 0.9cm acid treated Florisil. The silica and acid treated Florisil are separated from each other with a layer of filter paper. 1.3 gram concentrate obtained from the same manner as Example 1 are added to the top of this column. Feed to adsorbent ratio is 43g/kg. The concentrate consisted of 0.3% squalene. 3% carotene, 1% vitamin E, 0.6% sterols and 4% monoglycerides. Hexane:IPA (99.5:0.5) are added to the top of the column to desorb the minor components with pressure less than 1 bar. Four fractions are collected.

The whole run took less than 20 minutes. The first fraction is colorless consisted of 34% squalene with 80% recovery. The second fraction is red in color consisted of 83% carotene with 53% recovery while the third fraction being orangey in color consisted of 3% vitamin E with 87% recovery. The last fraction is colorless consisted of 2% sterols. After the last fraction is collected, residues in acid treated florisil are extracted using IPA. This residue consisted 30% monoglycerides.

Example 13

A 40mm I.D. column is packed with 6.8cm length normal phase silica and top up with 0.7cm polyethylene glycol. The silica and polyethylene glycol are separated from each other with a layer of filter paper. 1.3 gram concentrate obtained from the same manner as Example 1 are added to the top of this column. The concentrate consisted of 0.4% squalene. 3% carotene, 4% vitamin E, 0.1% sterols and 5% monoglycerides. Hexane:IPA (99.5:0.5) are added to the top of the column to desorb the minor components with pressure less than 1 bar. Five fractions are collected. The first fraction is colorless consisted of 30% squalene with 53% recovery. The second fraction is red in color consisted of 40% carotene with 80% recovery while the third fraction being orangey in color consisted of 10% vitamin E with 85% recovery. The fourth fraction is colorless consisted of 1% sterols with 35% recovery. The last fraction consisted of 75% diglycerides with 80% recovery. After the last fraction is collected, residues in polymer are extracted using IPA. This residue consists of 6% monoglycerides.

Example 14

A 40mm I.D. column is packed with 6.9cm length normal phase silica and top up with 0.6cm polyacrylate polyalcohol. The silica and polymer separated from each other with a layer of filter paper. 1.3 gram concentrate obtained from the same manner as Example 1 are added to the top of this column. The concentrate consisted of 0.4% squalene. 3% carotene, 4% vitamin E, 0.1% sterols and 5% monoglycerides. Hexane:IPA (99.5:0.5) are added to the top of the column to desorb the minor components with pressure less than 1 bar. Five fractions are collected. The first fraction is colorless consisted of 30% squalene with 70% recovery. The second fraction is red in color consisted of 34% carotene with 60% recovery while the third fraction being orangey in color consisted of 10% vitamin E with 60% recovery. The fourth fraction is colorless consisted of 0.7% sterols with 100% recovery and the last fraction consisted of 60% diglycerides with 90% recovery. After the last fraction is collected, residues in polymer are extracted using IPA. This residue consisted 30% monoglycerides.

Example 15

1.3g concentrate obtained from the same manner as Example 1 is dissolved in hexane and added to the top of a 7.5cm long. 4cm internal diameter silica column. Mixture of Hexane:IPA (99.5:0.5) is added to the top of the column with pressure less than 2 bar with flowrate 18ml/min to desorb the minor components. Fractions collected are determined with the following results:

	Squalene	Carotene	Tocols	Sterols
Concentration in feed, %	0.2	2.6	1.0	0.8
Concentration in fraction, %	10	95	3	2.0
Recovery, %	93	96	84	21

Example 16

1.5g concentrate obtained from the same manner as Example 1 is dissolved in hexane and added to the top of a 12cm long. 4cm internal diameter silica column. Mixture of Hexane:IPA (99.5:0.5) is added to the top of the column with pressure at least 0.5 bar to desorb the minor components. Fractions collected are determined with the following results:

	Squalene	Carotene	Tocols	Sterols
Concentration in feed, %	0.2	4.2	1.0	0.8
Concentration in fraction, %	10	67	14.0	1.4
Recovery, %	100	42	60	41

Example 17

0.5g concentrate obtained from the same manner as Example 1 is dissolved in dichloromethane and loaded to a 25cm length, 20cm internal diameter RP C18 column. Mixture of acetonitrile:DCM (89:11) is added to the column with pressure less than 1500psi to desorb the carotenes. Four fractions are collected based on their respective UV-Vis absorption maxima. The first fraction with UV-Vis abs max at 500nm is 98% lycopene with 80% recovery. Second fraction consisted of α-carotene while the third

fraction consisted of β-carotene. The last fraction collected with UV-Vis abs max at 286nm is 95% phytoene with 80% recovery.

Example 18

0.2g sterols-rich fraction obtained from the first extraction is dissolved in methanol and loaded to a 25 cm length, 20 mm internal diameter RP C18 column. Mixture of MeOH:H20:CH3COOH (98:1:1) is added to the column with pressure less than 1500psi to desorb the sterols. 5 fractions were collected in which the last fraction consisted of b-sitosterol with more than 98% purity. Recovery of the said compound is >60%.

While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purpose of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein can be varied considerably without departing from the basic principles of the invention.